Technical paper

Root and butt rot of orange trees in South-Western Nigeria

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Abstract — Introduction. In Nigeria, orange trees (*Citrus sinensis* (L.) Osb.) are being attacked by numerous diseases. One of them could be the root and butt rot disease caused by *Ganoderma pseudoferreum* (*G. philippii*). A study was conducted to identify the causal fungus and determine its pathogenicity in controlled conditions. **Materials and methods**. The agent of root and butt rot of orange trees was isolated from affected (dead), standing stems of orange plants, plating out on potato dextrose agar (PDA) medium and identified. Fungus growth was studied on PDA and malt extract agar (MEA). Pathogenicity tests were carried out on young seedlings of orange, guava (*Psidium guajava* L.) and papaya (*Carica papaya* L.) trees. **Results and discussion**. *Ganoderma pseudoferreum* (*G. philippii*) was identified as the fungus causing the disease. Within 17 d at room temperature (28 ± 2 °C), the fungus reached an averge of 5.12 ± 0.09 cm and 8.86 ± 0.03 cm in length on PDA and MEA, respectively. After 4 weeks of incubation, it was not reisolated in the control or in the experimental seedlings of orange and guava tree root sections; nevertheless, in papaya tree root sections, the rate of reisolation was 16.6 %. **Conclusion**. The fungus isolated from affected orange trees, which would be *G. pseudoferreum*, grows faster and best on malt extract agar. The control and experimental plants did not show symptoms of the disease after 4 weeks of incubation suggesting that the roots were not colonized by the fungus. The reason for the absence of symptom development has to be thoroughly investigated. © Éditions scientifiques et médicales Elsevier SAS

Nigeria / *Citrus sinensis* / pathogens / *Ganoderma pseudoferreum* / plant diseases / root rots / culture media / tissue culture

Pourridié basal et racinaire des orangers au sud-ouest du Nigeria.

Résumé — Introduction. Au Nigeria, les orangers (*Citrus sinensis* (L.) Osb.) sont attaqués par de nombreuses maladies. L'une d'elles pourrait être la maladie du pourridié basal et racinaire provoqué par *Ganoderma pseudoferreum* (*G. philippii*). Une étude a été entreprise pour identifier le champignon en cause et déterminer sa pathogénicité en conditions contrôlées. **Matériel et méthodes**. Le champignon responsable de la maladie a été isolé sur les tiges d'orangers affectés (morts), mis en culture sur un milieu gélifié à base de dextrose de pomme de terre (PDA), puis identifié. La croissance du champignon a été étudiée sur ce même milieu et sur de l'extrait de malt gélifié (MEA). Des tests de pathogénicité ont été effectués sur de jeunes orangers, goyaviers (*Psidium guajava L.*) et papayers (*Carica papaya*). **Résultats et discussion**. *Ganoderma pseudoferreum* (*G. philippii*) a été identifié comme étant le champignon responsable de la maladie. Après 17 d à la température ambiante (28 ± 2 °C), le champignon a atteint une longueur moyenne de 5,12 ± 0,09 cm et 8,86 ± 0,03 cm sur milieux PDA et MEA, respectivement. Après 4 semaines d'incubation, il n'a été réisolé ni dans les racines des plantules témoins, ni dans celles des jeunes orangers et goyaviers ; en revanche, il a été trouvé dans les racines de 16,6 % des jeunes papayiers inoculés. **Conclusion**. Le champignon isolé dans les orangers affectés, qui serait *G. pseudoferreum*, croît plus rapidement et mieux sur milieu MEA que sur PDA. Après 4 semaines d'incubation, les plantules inoculées n'ont pas exprimé les symptômes de la maladie, ce qui suggère que les racines n'ont pas été colonisées par le champignon. Les raisons de l'absence du développement de symptômes devront être étudiées de façon approfondie. © Éditions scientifiques et médicales Elsevier SAS

Nigeria / *Citrus sinensis* / agent pathogène / *Ganoderma pseudoferreum* / maladie des plantes / pourriture des racines / milieu de culture / culture de tissu

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1. introduction

Citrus species, probably originated in northeastern India, are cultivated on a small scale in West Africa. The cultivation of *Citrus* is not well organized and encouraged to maximize the use of the various products obtainable from the fruit [1]. *Citrus* species are grown principally for the juices of their fruit. After juice extraction, the fruit pulp is used for livestock feed and the rind acid (oil) is an expensive commodity in the international market. The seed of *Citrus* contains sweetening agents which are being considered as a probable substitute for sugar in the world market. Cultivated *Citrus* species grow well in Nigeria [1] as in both tropical and subtropical parts of the world provided that there is a sufficient moisture and that temperatures do not drop below freezing point [2].

A number of diseases have been found to be associated with *Citrus* species in Nigeria [1]. These probably include the root and butt rot caused by the fungus *Ganoderma pseudoferreum*, a basidomycete of the family Polyporaceae and the order Polyporales [3]. The natural habitat of the fungus is a variety of dead or dying trees [4]. The fungus occurs as an annual polypore which is found near the soil interface and, occasionally, on soils arising from buried roots [4]. This fungus often rots the roots of aged or diseased trees, causing them to fall. *G. lucidium* was reported causing rot on young *Citrus* [5] and *Phytopthora nicotianae* var. *parasitica* was associated with root rot [6]. Also, *Ganoderma pseudoferreum* (*G. philippii*) caused root rot in *Hevea brasiliensis* [7, 8] and cocoa [9]. Many workers have reported on the root and butt rot caused by *Heterobasidion annosum*. These diseases included root and butt rot of larch trees [10, 11], conifers [12] and spruce or *Picea abies* [13]. Root and butt rot were also caused by *Phaseolus schweinitzii* [10] and *Armillaria ostoyae* [14].

Several measures have been adopted to control root and butt rot diseases of plants caused by *H. annosum*. The most popular chemical control methods are the stump treatment of conifers with borate and urea [15] and the use of disodium octaborate tetrahydrate [16]. Control measures used for *Ganoderma pseudoferreum* diseases involved cutting trenches to isolate affected areas in rubber plantations [7] and inoculation of seedlings of cocoa with vesicular arbuscular fungi. [9]

In developing countries such as Nigeria, where root and butt rot diseases are causing havoc to citrus farms, it is important to identify the causal organisms and seek easy means of control. This paper, therefore, reports the isolation of *Ganoderma pseudoferreum* from mature, diseased orange tree roots and assesses its effect (pathogenicity) on young seedlings of orange (*Citrus sinensis* (L.) Osb.), guava (*Psidium guajava* L.) and papaya (*Carica papaya* L.) trees under greenhouse conditions.

2. materials and methods

2.1. fungus isolation

The fungus isolation was made from affected (dead) plants of *Citrus sinensis* L. in the ornamental farm of University of Benin, Nigeria. The tip of the fruiting body growing from the tree base was finely cut off with the aid of a sharp sterile razor blade and surface-sterilized in alcohol (70 % ethanol) for 1 min before plating out on potato dextrose agar (PDA) medium containing antibiotic mixture; there were three replicates. The plates were then incubated at room temperature (28 ± 2 °C) near a 5-ft fluorescent daylight tube for 3 d. Thereafter, the out growing fungus was isolated and identified as *Ganoderma pseudoferreum* using standard procedure [17, 18].

2.2. growth of *G. pseudoferreum*

Growth of *Ganoderma pseudoferreum* was measured in two media: PDA and malt extract agar (MEA). From a pure culture of *G. pseudoferreum*, inoculum discs (5 mm in diameter) were cut with a sterile cork borer and thereafter transformed and inoculated face-down on potato dextrose agar and malt extract agar at the centre of each

Petri-dish. Plates were incubated at room temperature (28 ± 2 °C) near a 5-ft fluorescent daylight tube. Measurement of the fungal growth was started 3 d after plating and incubation by inverting each plate and measuring the diameter of fungal mycelial growth daily for 7 d. The experiment was terminated when fungal mycelia on MEA made contact with the edge of the plates.

2.3. *G. pseudoferreum* tissue-block inoculum preparation

Living stems of an orange plant were cut and used for the *Ganoderma pseudoferreum* tissue-block experiment. The bark of the stems were properly peeled off and the stem finely cut into blocks of size 2 cm × 2 cm, with the aid of a sharp-edged knife. Five hundred pieces of these tissue blocks were washed, packed into a clean 1-L conical flask and autoclaved at 121 °C for 15 min. Thereafter, the sterile tissue blocks in the flask were left to cool and then inoculated with the mycelial suspension of *G. pseudoferreum*. The mycelial suspension was prepared by scrapping five (8 d old) colonies of the pure 5 cm in diameter fungus isolate into 100 mL of potato dextrose broth. The tissue-block and *G. pseudoferreum* mixtures were thoroughly shaken in the 1-L flask for 5 min. To provide moisture for the fungus, 50 mL of sterile water were added in the flask. The experiment was incubated at room temperature (28 ± 2 °C) for 3 weeks to allow the infection of the tissue-blocks by the fungus. The *G. pseudoferreum* tissue-blocks served as inoculum for the pathogenicity test.

2.4. fungus pathogenicity tests

Pathogenicity test of the fungus was carried out on orange (*Citrus sinensis*), guava (*Psidium guajava*) and papaya (*Carica papaya*) tree seedlings under greenhouse conditions. Two-weeks-old seedlings of orange, guava and papaya trees were transplanted from the field onto a bed of sterile loam soil, watered daily with sterile water and allowed to root for 2 months under greenhouse conditions. After 2 months, the seedlings were transplanted into sterile 10 cm × 5 cm cups filled with sterile loam soil and *G. pseudoferreum* tissue-blocks (ten tissue-blocks per cup), the ratio of soil to tissue-block being 2:1. Both the experimental and control treatments were placed in a greenhouse made of mosquito gauze netting in a well-lit corridor and watered daily with sterile water for 4 weeks.

2.5. lesion development on the seedling roots and fungus reisolation

At the end of these 4 weeks, the roots of the experimental and control plants (orange, guava and papaya) were closely examined for lesion (dead tissues) development. Thereafter, root sections of the experimental and control plants (2 mm in diameter) were cut, washed in 10 mL of sterile water in sterile McCartney bottles by hand shaking. The procedure was repeated ten times. Hereafter, the sections were surface sterilized in 70 % alcohol and finally rinsed with sterile water before plating on malt extract agar containing antibiotic mixture. There were six replicate sections per treatment (control and experimental seedlings). Plates were incubated at room temperature (28 ± 2 °C) near a 5-ft fluorescent daylight tube for 4 d to see if *G. pseudoferreum* was associated with the root sections.

3. results and discussion

The fungus *G. pseudoferreum* grew slowly on malt extract agar (MEA) and potato dextrose agar (PDA). Within 17 d, it reached 8.86 cm on MEA from an initial length of 1.104 cm, and 5.12 cm on PDA from an initial length of 0.44 cm (*figure 1*). The results showed an average daily increase of 1 cm in diameter of the fungus on MEA and 0.5 cm in diameter on PDA. These data agree with the previous findings [19] that the fungus has a slow rate of growth on PDA and that its growth is fairly rapid in MEA into which peptone has been added.

In this study, *G. pseudoferreum* was found to be non-pathogenic on the young

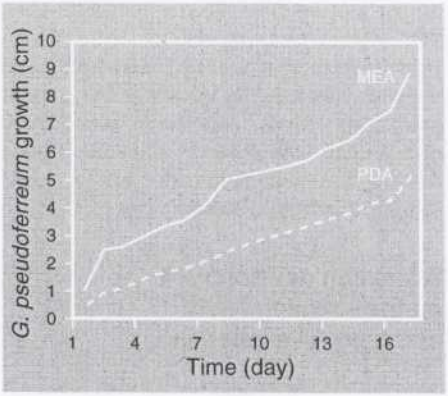

Figure 1.
Growth of *Ganoderma pseudoferreum* in Petri-dishes with malt extract agar (MEA) or potato dextrose agar.

seedlings of orange, guava and papaya trees. Neither the control plants nor the experimental ones showed disease symptoms after 4 weeks of incubation. Young seedlings of orange, papaya and guava trees would not be susceptible to *G. pseudoferreum* infection. However, 11 weeks after inoculation, 10 % infection of cocoa seedlings were caused by the fungus [9]. Ikediugwu (personal communication) observed that mature orange and guava trees were susceptible to *G. pseudoferreum* infection and that the fungus caused root and stem decay and, eventually, death of the trees. Also, in Bangladesh, in 1987, a root rot in *Hevea brasiliensis* plantation was reported to be caused by *G. pseudoferreum* which was controlled by cutting trenches to isolate affected areas [7]; in Malaysia, *G. pseudoferreum* was associated with the red root rot of rubber [8]. Yet, the results seems to agree with other findings [20], which found the fungus on stumps near the soil interphase and, occasionally, on soil arising from buried roots; this fungus often rotted the roots of aged or diseased trees causing them to fall. In this study, *G. pseudoferreum* was isolated, near the soil inter-phase, from affected (dead) standing stems of orange trees. This fungus was also reported causing heavy damage as the root and butt rot disease on living trees [21] and we observed similar damage on mature orange trees from which *G. pseudoferreum* was isolated. Thus, a better understanding of the fungus ecology is necessary for carrying out measures to control the disease in the future.

Table I.
Development of lesion and *Ganoderma pseudoferreum* reisolation rate (% of inoculated seedlings) on the roots of orange, guava and papaya tree seedlings.

Treatment	Number of lesions	*G. pseudoferreum* reisolation rate (%)
Control: sterile soil		
orange seedlings	0	0[1]
papaya seedlings	0	0
guava seedlings 0 0		
Experimental: sterile soil + *G. pseudoferreum* tissue-block		
orange seedlings	0	0
guava seedlings	0	0
papaya seedlings	0	16.6

[1] When *G. pseudoferreum* was not reisolated, the reisolation rate was zero.

In this study, *G. pseudoferreum* did not cause root decay in young seedlings of orange, papaya and guava trees. Nevertheless, this result does not agree with a previous report according to which the fungus would kill young trees by decaying the root and root collar wood; resistance to killing would increase with age and would be strong from about 25 years upwards [22]. Similarly, *G. pseudoferreum* caused root rot infection on young orange trees growing in USA [5].

In our work, studies on soil pH and other factors were not carried out. In the root and butt rot of Japanese larch tree, the environmental conditions such as soil moisture and soil pH contributed to the development of the disease in larch forest [11].

The fungus *G. pseudoferreum* was not reisolated in all the control (uninoculated) seedling root sections (orange, guava and papaya), nor in the experimental (inoculated) root sections of orange and guava seedlings. However, 16.6 % reisolation of *G. pseudoferreum* was obtained in the experimental papaya tree root seedlings (*table I*). The low level of *G. pseudoferreum* reisolation from the seedlings may be due to the non-colonization of the root by the fungus within 4 weeks of incubation, but the 16.6 % success recorded with the papaya tree seedlings suggests that the fungus could actually colonize the roots, but

this colonization would most probably occur over a long period of time during which the fungus would be inactive in the soil.

4. conclusion

Growth of *G. pseudoferreum* on malt extract agar and potato dextrose agar was slow in both media, although this growth was faster and better on malt extract agar. Under greenhouse conditions, the control and experimental plants did not show symptoms of disease after 4 weeks of incubation. The period of incubation might have been too short for developing disease symptoms or, most probably, might be due to the fact that the seedling roots had not been colonized by the fungus. However, the reasons for the absence of symptom development is not discernable for the observations made.

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Podredumbre basal y de las raices del naranjo en el sudoeste de Nigeria.

Resumen — Introducción. En Nigeria, numerosas enfermedades atacan los naranjos (*Citrus sinensis* (L.) Osb.). Una de ellas podría ser la enfermedad podredumbre basal y de las raices causada por *Ganoderma pseudoferreum* (*G. philippii*). Un estudio fue conducido para identificar el hongo causal y para determinar su patogenicidad en condiciones controladas. **Material y métodos**. El agente de la enfermedad fue aislado sobre ramas de naranjos afectados (muerto), puesto en medio de cultivo con un gel de la dextrosa de la patata (PDA), e identificado. El crecimiento del hongo fue estudiado en PDA y en extracto de malta (MEA). Las pruebas del patogenicidad fueron realizadas en semillero sobre plantas jóvenes de naranjo, guayaba (*Psidium guajava* L.) y papaya (*Carica papaya*). **Resultados y discusión**. *Ganoderma pseudoferreum* (*G. philippi*) fue identificado como el hongo que causaba la enfermedad. En 17 días a temperatura ambiente (28 ± 2 °C), el hongo alcanzó 5,12 ± 0,09 cm y 8,86 ± 0,03 cm en longitud en PDA y MEA, respectivamente. Después de 4 semanas de incubación, no fue reaislado en las raíces de las plantas testigo así como en los naranjos y guayabos ; sin embargo, fue encontrado en las raíces de 16,6 % de los de papayos inoculados. **Conclusión**. El hongo aislado de los naranjos afectados, que sería *Ganoderma pseudoferreum*, crece más rápidamente y mejor en un medio de extracto de malta. El control y las plantas inoculadas no mostraron los síntomas de las enfermedades después de 4 semanas de incubación que sugerían que las raíces no fueron colonizadas por el hongo. La razón de la ausencia del desarrollo de los síntomas tiene que ser más investigada.

Nigeria / *Citrus sinensis* / organismos patógenos / *Ganoderma pseudoferreum* / enfermedades de las plantas / podredumbre de la raíz / medio de cultivo / cultivo de tejidos